Catchment topography and the distribution of electron donors for denitrification control the concentration of nitrate in headwater streams of the Lake Hachiro watershed

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Abstract

We examined the linkages between topography and electron donors for denitrification in headwater streams in the Lake Hachiro watershed that has marine sedimentary rocks. In 33 headwater streams, we sampled water at the catchment nine times in 2 years. Stream sediment was sampled once for measurement of denitrification potential (DP), water-extractable soil organic carbon (WESOC), and easily oxidizable sulfide (EOS), which are considered the principal potential electron donors for denitrification. The topographical features of each catchment were calculated using a digital elevation model with 10-m grid cells. Stream NO_3^- concentrations displayed large spatial variation among catchments, ranging from 0.06 to 0.52 mg N L^{-1}, and were significantly positively correlated with slope in the catchments (r = 0.663, P < 0.01, n = 33), indicating that NO_3^- was removed to a greater extent in gentle slope catchments. Generalized linear model showed slope, slope aspect, sediment DP, and EOS significantly affected in-stream NO_3^- concentration. Stream SO_4^{2-} concentrations tended to increase as NO_3^- concentrations decreased and EOS contents increased, indirectly indicating sulfur-mediated denitrification. NO_3^- reduction with SO_4^{2-} production, and sulfur-oxidizing bacteria was detected in the stream bank soil with high EOS content. We conclude that catchment topography and the distribution of electron donors in riverbed sediment explain the spatial variation in in-stream NO_3^- concentration and, by inference, catchment denitrification. These results indicate that more NO_3^- may be denitrified by sulfur-mediated denitrification owing to the abundance of sulfides in the catchment from marine sedimentary rocks.

Key Words

denitrification, hydrogeology, riparian, sulfur denitrification, topography

Introduction

Denitrification involves the conversion of nitrate (NO_3^-) into gaseous forms of N, generally by particular groups of ubiquitous heterotrophic bacteria that have the ability to use NO_3^- as an electron acceptor and carbon (C) as an electron donor during anaerobic respiration. Human activities dramatically increased the amount of reactive N in global ecosystems (Galloway and Cowling 2002); however, the mass balance approach in watersheds frequently fails to account for a large proportion of the N, and this "missing N" is considered to represent the fraction that undergoes transfer to the atmosphere by denitrification (Howarth et al. 1996).

Landscape topography can control biogeochemical cycles such as denitrification. At sites with a relatively flat topography, the low hydraulic gradient increases water residence time in the riparian zone and enhances the development of anaerobic conditions necessary for denitrification (Vidon and Hill 2004). Despite much effort and many studies at plot and microtopographic scales showing the effects of elevation and topography on soil denitrification potential (DP) within riparian forests, limited research has been conducted at broader landscape scales.

Generally, C is known as the primary electron donor for denitrification in ecosystems. But electron donors other than C can also drive denitrification. Some bacteria can use inorganic sources, such as reduced sulfur to grow chemoautotrophically (Korom 1992). NO_3^- reduction coupled with sulfide oxidation could be widespread and biogeochemically important in freshwater sediments (Burgin et al., 2008), however, the relative importance of the electron donor in the removal process remains uncertain at watershed scales.

Lake Hachiro in Akita prefecture, Japan, was designated in 2007 under the Law Concerning Special Measures for Conservation of Lake Water Quality in Japan because it is eutrophic and the need to improve its water quality is considered urgent. One of the special characteristics of the Lake Hachiro watershed (LHW) is its geology. The region was submerged beneath the sea during the Neogene period (Shiraishi and Matoba 1992), so sulfide minerals are expected to be distributed throughout the watershed. High sulfide content could be reasonably expected to influence the N cycle through sulfur-driven denitrification. The hypothesis of this study was that the interaction between topographic factors and availability of electron donors for denitrification will control the amount of denitrification at the catchment scale. To test this hypothesis, we evaluated the

effects of catchment topography and amounts of organic C and reduced sulfur in riverbed sediments (a proxy for denitrification) on in-stream NO_3^- concentration in addition to measured riverbed sediment denitrification potential in 33 headwater catchments of the LHW, Akita prefecture, Japan.

Methods

Site description

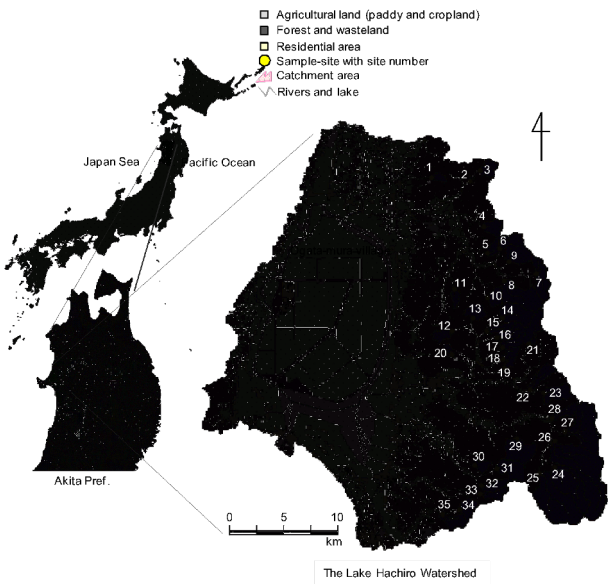

Figure 1. Location of the Lake Hachiro watershed (LHW), Akita, Japan, and the land-use distribution and location of studied headwater catchments and sampling sites within the watershed.

The LHW is located in western Akita prefecture facing the Japan Sea (Fig. 1). The entire watershed area is 894 km^2. Precipitation averages 1553 mm year^{-1} and the annual mean temperature is 10.8°C. The lowest mean monthly temperature occurs in January (–0.9°C) and the highest mean monthly temperature in August (24°C). The average maximum snow depth during winter (December–February) is 48 cm.

The geological strata of the watershed belong to the Green Tuff zone, consisting of volcanic rocks and sedimentary rocks of the later Miocene (Shiraishi 1990). In the early Middle Miocene, the Akita region was drowned as a result of subsidence of previous land areas. The Oga Peninsula, where Lake Hachiro is located, was under deep water in the Early to Middle Pleistocene and later became a shallow marine shelf in the late to Middle Pleistocene. As a result, the sedimentary rocks in the LHW are mainly marine deposits and comprise thick mudstone layers (Shiraishi 1990).

We established sample sites for the determination of water quality at positions that then defined the outlet of 33 headwater catchments of the five main rivers entering Lake Hachiro (Fig. 1). Each catchment was independent and all were forested. The geography and topography of each catchment were characterized using a DEM of 10 m × 10 m resolution using the GIS software TNTmips (Microimages Inc., Lincoln, NE, USA).

Water, sediment, and soil sampling

Stream water samples were collected nine times—December 2009 and May, July, September, and December of 2010 and 2011—from the 33 sample sites in the LHW. Sediment samples from the riverbed of each sample site were obtained once in July 2011 at the same time as water sampling. We collected the samples from the 0–5 cm depth at over 10 random locations from the riverbed at the sample site and sieved them to under 2.0 mm on site, and then combined the samples into a bulked sample for analysis. The riverbed sulfur content was relatively high at site No. 15, so in February 2014 and September 2015 soil samples were collected from a vertical stream bank.

Analysis of water, sediment, and soil samples

The water samples were filtered through a 0.45-µm membrane filter. We measured the NO_3^- and SO_4^{2-} concentrations in the water using an ion chromatograph. The fresh sediment and soil samples were extracted in distilled water (soil:water, 1:5, w/v), and the concentrations of WESOC was determined using a total organic C analyzer (TOC-5000, Shimadzu, Kyoto, Japan). Easily oxidizable-sulfur (EOS) content in the sediment and soil samples was determined by the difference between H_2O_2-soluble sulfur (H_2O_2-S) and water-soluble sulfur (H_2O-S) contents.

The denitrification potential (DP) of sediment samples was measured to quantify the variation among sites in the amount of electron donors available to denitrifying organisms. We defined DP as the denitrification rate that occurred under anaerobic conditions with abundant NO_3^- at 25°C and measured it using acetylene inhibition assay, which inhibits the final step in the conversion of N_2O gas into N_2 gas.

DNA was extracted from the stream bank soils, and prokaryotic communities were analyzed using polymerase chain reaction (PCR) for 16S rRNA genes. Sequencing determined using Miseq system (Illumina).

Results and discussion

Spatial variations of in-stream NO_3^- concentration, sediment DP, and potential electron donors for denitrification in riverbed sediments.

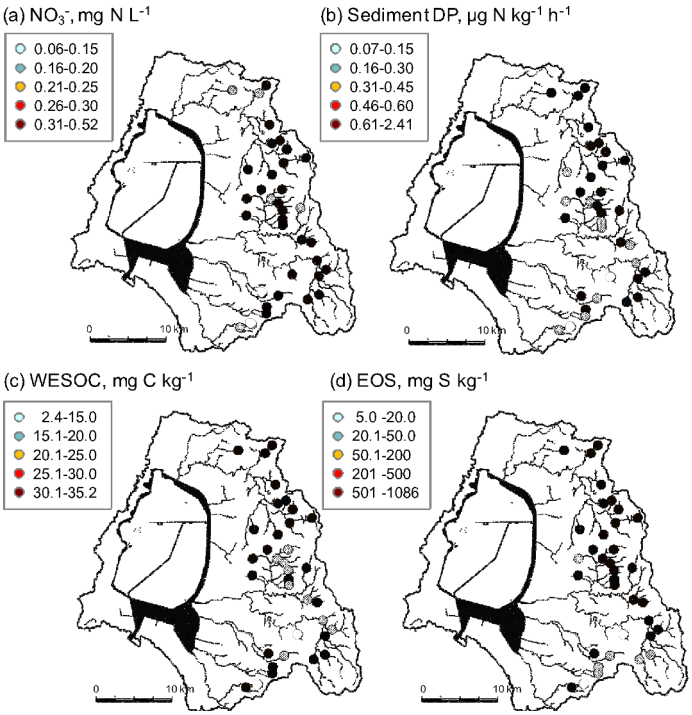

Figure 2. Spatial distribution of (a) NO_3^- concentration, (b) denitrification potential (DP) of riverbed sediment, (c) water-extractable carbon (WESOC) content of riverbed sediment, and (d) easily oxidizable sulfur (EOS) content of riverbed sediment at the outlets of headwater catchments of the Lake Hachiro watershed.

Mean NO_3^- concentrations in the headwater streams displayed large spatial variation (Fig. 2a), ranging from 0.06 to 0.52 mg N L^{-1}. Mean in-stream NO_3^- concentration at the catchment outlets was strongly correlated with the catchment topographic factors of median slope (r = 0.66, P < 0.01, n = 33), median slope aspect (r = 0.48, P < 0.01, n = 33). The sediment DP, WESOC and EOS also displayed large spatial variation (Fig. 2). Sediment DP and WESOC tended to be higher (Fig. 2b, c) in the northern part of the watershed. In-stream NO_3^- concentration was strongly correlated with the sediment DP (r = -0.52, P < 0.01, n = 33) and WESOC (r = -0.53, P < 0.01, n = 33). EOS content varied greatly among the catchments, ranging from 5.0 to 1086 mg S kg^{-1}, and displayed the highest values in the middle and northern part of the watershed (Fig. 2d).

Generalized linear models analysis

Generalized linear models (GLMs) with a log link function were used to test for the effect of geographical and topographical conditions, riverine physical and chemical conditions in catchments on in-stream NO_3^- concentration using R ver 2.12.2 (R Development Core Team, 2011). The results showed sediment DP, median slope, median slope aspect, latitude, sediment EOS, catchment size, and pH were identified as significant predictors of NO_3^- concentration (Table 1).WESOC did not selected as the explanatory variable likely because it correlated median slope significantly (r = -0.65, P < 0.01, n = 33).

Possibilities for sulfur denitrification in the sulfide rich catchments

The GLM showed the sediment EOS was one of the significant predictor of NO_3^- concentration (Table 1). Stream NO_3^- concentration tended to decrease (r = –0.27, P = 0.13, n = 33) with EOS content, and SO_4^{2-} concentration increased significantly with EOS content (r = 0.71, P < 0.01, n = 33). These results indirectly indicated sulfur-mediated denitrification would occur in the catchment that has higher EOS in sediments.

At site No. 15 the riverbed EOS content was relatively high at 254 mg kg^{-1} (Fig. 2d) and therefore the soil in a stream bank was vertically sampled. The vertical distribution of WESOC content was highest at the surface (333 mg kg^{-1}), intermediate at 50 cm, and reached a low constant value of about 80 mg kg^{-1} at 75–200 cm. The EOS content was low and relatively constant from the surface to 160 cm but was extremely high (890 mg kg^{-1}) at 180–210 cm (Figure 3), in which NO_3^- reduction with SO_4^{2-} production was observed (Figure 4). Furthermore, as result of prokaryotic communities analysis, denitrifying sulfur oxidizing bacteria (*Thiobacillus denitrificans*) were detected from the sediment.

Conclusion

The present study demonstrated that catchment topography was the primary factor explaining the spatial variation of in-stream NO_3^- concentration in headwater catchments of the Lake Hachiro watershed (LHW) through its influence on water residence time and, by inference, denitrification. Electron donors for denitrification such as carbon and sulfur in riverbed sediments explain ecosystem-wide denitrification, indicated by in-stream NO_3^- concentration. Reduced sulfur in the sediment varied considerably among the

catchments, we hypothesis sulfur is coupled strongly with NO_3^- removal by promoting sulfur-mediated denitrification.

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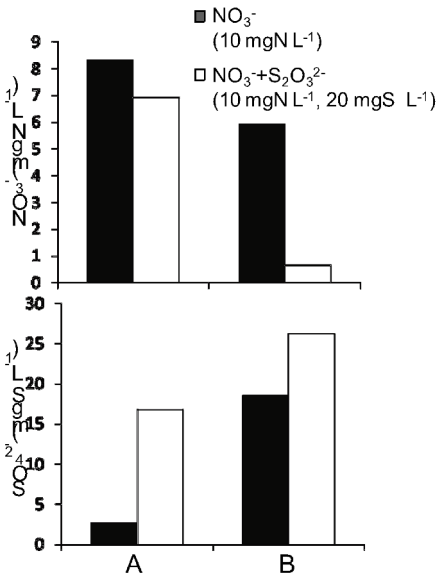
Table 1. Results of a generalized linear model (GLM) for explaining the variance in-stream NO_3^- concentration with explanatory variables comprising catchment physical conditions and sediment properties (n=33). The modelling was conducted using a GLM with a stepwise selection based on Akaike's information criterion (AIC).

Response variable	Explanatory variable	Estimate	±	SE	*t* value	*P* value	AIC
NO_3^- concentration	Intercept	-7.43E+01	±	2.25E+01	-3.30	0.003	-115
	Sediment DP	-4.61E-01	±	9.43E-02	-4.89	<0.0001	
	Median slope	7.29E-02	±	9.71E-03	7.50	<0.0001	
	Median slope aspect	5.46E-03	±	1.57E-03	3.48	0.002	
	Latitude	1.80E+00	±	5.59E-01	3.22	0.004	
	Sediment EOS	-4.60E-04	±	1.64E-04	-2.81	0.009	
	Catchment size	-2.90E-02	±	9.89E-03	-2.93	0.007	
	pH in streamwater	-2.50E-01	±	9.92E-02	-2.53	0.018	



igure 4. NO_3^- and SO_4^{2-} concentration fter one month incubation of soil with ifferent solution. A; soil above the sulfide-ich layer, B; soil in the sulfide rich layer in vertical stream bank at sample site No. 5.